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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	John Babish, et al.
Appl. No.	:	09/885,721
Filed	:	June 20, 2001
For	:	COMPLEX MIXTURES EXHIBITING SELECTIVE INHIBITION OF CYCLOOXYGENASE-2
Examiner	:	Michael V. Meller

Group Art Unit 1654

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: United States Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202, on

June 9, 2003
(Date)

Connie C. Tong, Reg. No. 52,292

DECLARATION UNDER 37 C.F.R. § 1.131

United States Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

We, John G. Babish and Terrence M. Howell, do hereby declare and say as follows:

1. We are the named joint inventors of the subject matter of patent Application Serial No. 09/885,721. All work described hereinafter was performed by us or on our behalf in the United States of America.

2. We have read the Office Action dated January 31, 2003 rejecting claims over, among other references, Newmark et al. (U.S. Patent No. 6,391,346). The filing date of the application that resulted in the Newmark patent is April 5, 2001. We have also reviewed the Amendment accompanying this Declaration.

3. We conceived the subject matter of all the pending claims, as presently amended, of the application prior to April 5, 2001 and were diligently working to reduce the claimed

invention to practice by conducting additional experiments and drafting the patent application. Therefore, we are entitled to an invention date prior to the filing date of Newmark et al.

4. Exhibit A shows a draft of the disclosure of the present patent application, the date on which has been redacted, but is dated before April 5, 2001. As explained in greater detail below, the draft of the patent application discloses all of the features in all of the pending claims. Accordingly, this document establishes our conception of the presently claimed invention prior to April 5, 2001.

5. Claim 1 recites "A composition for inhibiting inducible COX-2 activity, comprising a pharmaceutical grade CO_2 extract of hops and a pharmaceutically acceptable carrier; wherein said composition is formulated into a form selected from the group consisting of capsule, tablet, injectable solution, injectable suspension, spray solution, spray suspension, and lotion." Support for our conception for the subject matter of this claim can be found in Exhibit A. On page 2 of Exhibit A, the field of the invention states that "the present invention relates generally to a natural composition exhibiting specific inhibition of inducible cyclooxygenase-2 (COX-2). More particularly, the composition comprises an extract of hops (Humulus lupulus)." On page 10 of Exhibit A, it is disclosed that "pharmaceutical grade extracts are particularly preferred." On page 12 of Exhibit A, it is disclosed that the composition can be formulated into a capsule or tablet. Other forms include "injectable solution or suspension, a spray solution or suspension, a lotion..."

6. Claim 6 recites "The composition of Claim 1 formulated in a pharmaceutically acceptable carrier." Claim 15 recites "The composition of Claim 9 formulated in a pharmaceutically acceptable carrier." Support for our conception for the subject matter of these claims can be found in Exhibit A, page 11 which discusses a "pharmaceutically acceptable carrier." The disclosure further states that "Except insofar as any conventional media or agent is incompatible with the active ingredients, its use in the present composition is contemplated."

7. Claim 7 recites "The composition of Claim 1, further comprising one or more members selected from the group consisting of antioxidants, vitamins and minerals." Claim 16 recites "The composition of Claim 9, further comprising one or more members selected from the

group consisting of antioxidants, vitamins and minerals." Support for our conception for the subject matter of these claims can be found in Exhibit A, page 11 which states "the present composition for dietary application may include various additives such as other natural components of intermediary metabolism, vitamins and minerals..." Furthermore, since certain vitamins can act as antioxidants, the recitation of antioxidants in these claims is also supported.

8. Claim 8 recites "The composition of Claim 1, further comprising one or more members selected from the group consisting of proteins, fats, carbohydrates, glucosamine, chondrotin sulfate and amino sugars." Claim 17 recites "The composition of Claim 9, further comprising one or more members selected from the group consisting of proteins, fats, carbohydrates, glucosamine, chondrotin sulfate and amino sugars." Support for our conception for the subject matter of these claims can be found in Exhibit A, pages 8 and 11. On page 11 of Exhibit A, it is disclosed that "Other ingredients known to affect the manufacture of this composition as a dietary bar or functional food can include flavorings, sugars, amino-sugars, proteins and/or modified starches, as well as fats and oils." On page 8 of Exhibit A, it is disclosed that "the present invention further provides a composition of matter that enhances the function of glucosamine or chondrotin sulfate to normalize joint movement or reduce the symptoms of osteoarthritis."

9. Claim 9 recites "The composition of Claim 1, wherein the pharmaceutical grade CO_2 extract of hops comprises 30 to 60 percent alpha acids and 15 to 45 percent beta acids." Support for our conception for the subject matter of this claim can be found in Exhibit A, pages 4 and 13. On page 13 of Exhibit A, it is disclosed that a preferred composition is a CO_2 extract of hops. Table 1 on page 4 of Exhibit A shows that liquid CO_2 comprises 30-60 percent alpha acids and 15-45 percent beta acids.

10. Exhibit B shows pages from lab notebooks for the testing of a hops powder. The notebook pages show diligence towards working on experiments to actually reduce the claimed invention to practice in the time period of April 9, 2001 to April 16, 2001. Exhibit B documents testing of a hops powder and other compositions for the effectiveness for the inhibition of PGE_2. These compositions were intended for formulation into the various forms recited in Claim 1.

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11. Exhibit C shows a redacted copy of a telephone log of John G. Babish. The redacted material is relates to parties and commercial matters unrelated to the present patent application. The telephone log documents efforts in diligence towards working on experiments to actually reduce the claimed invention to practice and constructively reducing the invention to practice by filing the present patent application in the time period of May 2, 2001 to June 20, 2001. Exhibit C documents work towards developing a powder form of a hops extract, which is a form that can be delivered as a pharmaceutical composition, as presently claimed. Efforts towards developing a powder form of a hops extract were done in collaboration with a potential business partner. In the same time period, drafting of the patent application was done by one of the inventors and the patent counsel.

We declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true. We declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

6-6-03
Date

John G. Babish

6.6.03
Date

Terrence M. Howell

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UNITED STATES PATENT APPLICATION
FOR
AN EXTRACT FROM HOPS (*Humulus lupulus*) AS A SPECIFIC INHIBITOR OF CYCLOOXYGENASE-2 MEDIATED SYNTHESIS OF PROSTAGLANDINS

THE COMMISSIONER OF PATENTS AND TRADEMARKS:

Your petitioners, **JOHN G. BABISH**, a citizen of the United States, residing at 508 White Church Rd., Brooktondale, New York 14817; M. **LISA STRASSHEIM-LEE,** a citizen of the United States, residing at 349 Willow Crossing, Dryden, New York 13053; and **TERRENCE HOWELL**, a citizen of the United States, residing at 62 Southworth Road, Dryden, New York 13053 pray that letters patent may be granted to them as inventors of the improvement in **AN EXTRACT FROM HOPS (*Humulus lupulus*) AS A SPECIFIC INHIBITOR OF CYCLOOXYGENASE-2 MEDIATED SYNTHESIS OF PROSTAGLANDINS** as set forth in the following specification:

Exhibit A

FIELD OF THE INVENTION

The present invention relates generally to a natural composition exhibiting specific inhibition of inducible cyclooxygenase-2 (COX-2). More particularly, the composition comprises an extract of hops (*Humulus lupulus*). The complex composition functions to inhibit the inducibility and/or activity of inducible cyclooxygenase (COX-2) with little or no significant effect on constitutive cyclooxygenase (COX-1).

BACKGROUND OF THE INVENTION

Inflammatory diseases affect more than fifty million Americans. As a result of basic research in molecular and cellular immunology over the last ten to fifteen years, approaches to diagnosing, treating and preventing these immunologically-based diseases has been dramatically altered. One example of this is the discovery of an inducible form of the cyclooxygenase enzyme. Constitutive cyclooxygenase (COX), first purified in 1976 and cloned in 1988, functions in the synthesis of prostaglandins (PGs) from arachidonic acid.(AA) Three years after its purification, an inducible enzyme with COX activity was identified and given the name COX-2, while constitutive COX was termed COX-1.

COX-2 gene expression is under the control of pro-inflammatory cytokines and growth factors. Thus, the inference is that COX-2 functions in both inflammation and control of cell growth. While COX-2 is inducible in many tissues, it is present constitutively in the brain and spinal cord, where it may function in nerve transmission for pain and fever. The two isoforms of COX are nearly identical in structure but have important differences in substrate and inhibitor selectivity and in their intracellular locations. Protective PGs, which preserve the integrity of the stomach lining and maintain normal renal function in a compromised kidney, are synthesized by COX-1. On the other hand, PGs synthesized by COX-2 in immune cells are central to the inflammatory process.

The discovery of COX-2 has made possible the design of drugs that reduce inflammation without removing the protective PGs in the stomach and

kidney made by COX-1. Combinations of the invention would be useful for, but not limited to, the treatment of inflammation in a subject, and for treatment of other inflammation-associated disorders, such as, as an analgesic in the treatment of pain and headaches, or as an antipyretic for the treatment of fever. For example, combinations of the invention would be useful to treat arthritis, including but not limited to rheumatoid arthritis, spondyloathopathies, gouty arthritis, osteoarthritis, systemic lupus erythematosus, and juvenile arthritis. Such combination of the invention would be useful in the treatment of asthma, bronchitis, menstrual cramps, tendonitis, bursitis, and skin related conditions such as psoriasis, eczema, burns and dermatitis. Combinations of the invention also would be useful to treat gastrointestinal conditions such as inflammatory bowel disease, Crohn's disease, gastritis, irritable bowel syndrome and ulcerative colitis and for the prevention or treatment of cancer such as colorectal cancer. Combinations of the invention would be useful in treating inflammation in such diseases as vascular diseases, migraine headaches, periarteritis nodosa, thyroiditis, aplastic anemia, Hodgkin's disease, sclerodma, rheumatic fever, type I diabetes, myasthenia gravis, multiple sclerosis, sacoidosis, nephrotic syndrome, Behchet's syndrome, polymyositis, gingivitis, hypersensitivity, swelling occurring after injury, myocardial ischemia and the like.

The compounds would also be useful in the treatment of ophthalmic diseases, such as retinopathies, conjunctivitis, uveitis, ocular photophobia, and of acute injury to the eye tissue. The compounds would also be useful in the treatment of pulmonary inflammation, such as that associated with viral infections and cystic fibrosis. The compounds would also be useful for the treatment of certain nervous system disorders such as cortical dementias including Alzheimer's disease. The combinations of the invention are useful as anti-inflammatory agents, such as for the treatment of arthritis, with the additional benefit of having significantly less harmful side effects. As inhibitors of cyclooxygenase-2 mediated biosynthesis of PGE2, these compositions would also be useful in the treatment of allergic rhinitis, respiratory distress syndrome,

endotoxin shock syndrome, atherosclerosis, and central nervous system damage resulting from stroke, ischemia and trauma.

Besides being useful for human treatment, these compounds are also useful for treatment of other animals, including horses, dogs, cats, birds, sheep, pigs, etc. An ideal formulation for the treatment of inflammation would inhibit the induction and activity of COX-2 without affecting the activity of COX-1. Historically, the non-steroidal and steroidal anti-inflammatory drugs used for treatment of inflammation lack the specificity of inhibiting COX-2 without affecting COX-1. Therefore, most anti-inflammatory drugs damage the gastrointestinal system when used for extended periods. Thus, new COX-2 specific treatments for inflammation and inflammation-based diseases are urgently needed.

GENERAL INFORMATION ON PHARMACOLOGICAL EFFECTS OF HOPS NEEDED HERE.

Hop extraction in one form or another goes back over 150 years to the early nineteenth century when extraction in water and ethanol was first attempted. Even today an ethanol extract is available in Europe but by far the predominant extracts are organic solvent extracts (hexane) and CO2 extracts (supercritical and liquid). CO2 (typically at 60 bars pressure and 5 to 10°C) is in a liquid state and is a relatively mild, non-polar solvent highly specific for hop soft resins and oils. Beyond the critical point, typically at 300 bars pressure and 60°C, CO2 has the properties of both a gas and a liquid and is a much stronger solvent. The composition of the various extracts is compared in Table 1.

Table 1. Hop Extracts (Percent W/W)

Component	Hops	Organic Solvent Extract	Super-Critical CO2	Liquid CO2
Total resins	12 - 20	15 - 60	75 - 90	70 - 95
Alpha-acids	2 - 12	8 - 45	27 - 55	30 - 60
Beta-acids	2 - 10	8 – 20	23 - 33	15 - 45
Essential oils	0.5 – 1.5	0 - 5	1 - 5	2 - 10
Hard resins	2 - 4	2 - 10	5 - 11	none
Tannins	4 - 10	0.5 - 5	0.1 - 5	none

Waxes	1 - 5	1 - 20	4 - 13	0 - 10
Water	8 - 12	1 - 15	1 - 7	1 - 5

At its simplest, hop extraction involves milling, pelleting and re-milling the hops to spread the lupulin, passing a solvent through a packed column to collect the resin components and finally, removal of the solvent to yield a whole or "pure" resin extract.

The main organic extractants are strong solvents and in addition to virtually all the lupulin components, they extract plant pigments, cuticular waxes, water and water-soluble materials.

Supercritical CO2 is more selective than the organic solvents and extracts less of the tannins and waxes and less water and hence water-soluble components. It does extract some of the plant pigments like chlorophyll but rather less than the organic solvents do. Liquid CO2 is the most selective solvent used commercially for hops and hence produces the most pure whole resin and oil extract. It extracts none of the hard resins or tannins, much lower levels of plant waxes, no plant pigments and less water and water soluble materials.

As a consequence of this selectivity and the milder solvent properties is that the absolute yield of liquid CO2 extract per unit weight of hops is less than the other solvents. Additionally, the yield of alpha acids with liquid CO2 (89 93%) is lower than that of supercritical CO2 (91 – 94%) or the organic solvents (93 – 96%). Following extraction there is the process of solvent removal, which for organic solvents involves heating to cause volatilization. Despite this, trace amounts of solvent do remain in the extract. The removal of CO2, however, simply involves a release of pressure to volatilize the CO2.

PRIOR ART ON HOPS EXTRACTS -

Prior art describes the identification of humulone from hops extract as an inhibitor of bone resorption [Tobe, H. et al. 1997. Bone resorption Inhibitors from hop extract. Biosci. Biotech. Biochem 61(1)158-159]. Later studies by the same group characterized the mechanism of action of humulone as inhibition of COX-2

gene transcription following TNFalpha stimulation of MC3T3 –E1 cells [Yamamoto, K. 2000. Suppression of cyclooxygenase-2 gene transcription by humulon of bee hop extract studied with reference to glucocorticoid. FEBS Letters 465:103-106].

Thus, it would be useful to identify a natural formulation of compounds that would specifically inhibit or prevent the synthesis of prostaglandins by COX-2 with little or no effect on COX-1. Such a formulation would be useful for preserving the health of joint tissues, for treating arthritis or other inflammatory conditions has not yet been discovered. The terms specific or selective COX-2 inhibitor embrace compounds or formulations of compounds that selectively inhibit COX-2 over COX-1. Preferably, the compounds have a median effective concentration for COX-2 inhibition that is minimally five times greater than the median effective concentration for the inhibition of COX-1. For example, if the median inhibitory concentration for COX-2 of a test formulation was 0.2 µg/mL, the formulation would not be considered COX-2 specific unless the median inhibitory concentration for COX-1 was equal to or greater than 1 µg/mL.

While glucosamine is generally accepted as being effective and safe for treating osteoarthritis, medical intervention into the treatment of degenerative joint diseases is generally restricted to the alleviation of its acute symptoms. Medical doctors generally utilize non-steroidal and steroidal anti-inflammatory drugs for treatment of osteoarthritis. These drugs, however, are not well adapted for long-term therapy because they not only lack the ability to promote and protect cartilage; they can actually lead to degeneration of cartilage or reduction of its synthesis. Moreover, most non-steroidal, anti-inflammatory drugs damage the gastrointestinal system when used for extended periods. Thus, new treatments for arthritis are urgently needed.

The joint-protective properties of glucosamine would make it an attractive therapeutic agent for osteoarthritis except for two drawbacks: (1) the rate of response to glucosamine treatment is slower than for treatment with anti-inflammatory drugs, and (2) glucosamine may fail to fulfill the expectation of degenerative remission. In studies comparing glucosamine with non-steroidal

anti-inflammatory agents, for example, a double-blinded study comparing 1500 mg glucosamine sulfate per day with 1200 mg ibuprofen, demonstrated that pain scores decreased faster during the first two weeks in the ibuprofen patients than in the glucosamine-treated patients. However, the reduction in pain scores continued throughout the trial period in patients receiving glucosamine and the difference between the two groups turned significantly in favor of glucosamine by week eight. Lopes Vaz, A., Double-blind clinical evaluation of the relative efficacy of ibuprofen and glucosamine sulphate in the management of osteoarthritis of the knee in outpatients, 8 Curr. Med Res Opin. 145-149 (1982). Thus, glucosamine may relieve the pain and inflammation of arthritis at a slower rate than the available anti-inflammatory drugs.

An ideal formulation for the normalization of cartilage metabolism or treatment of osteoarthritis would provide adequate chondroprotection with potent anti-inflammatory activity. The optimal dietary supplement for osteoarthritis should enhance the general joint rebuilding qualities offered by glucosamine and attenuate the inflammatory response without introducing any harmful side effects. It should be inexpensively manufactured and comply with all governmental regulations.

However, the currently available glucosamine formulations have not been formulated to optimally attack and alleviate the underlying causes of osteoarthritis and rheumatoid arthritis. Moreover, as with many commercial herbal and dietary supplements, the available formulations do not have a history of usage, nor controlled clinical testing, which might ensure their safety and efficacy.

It would be useful to identify a formulation of compounds that would specifically inhibit or prevent the expression of COX-2 enzymatic activity, while having little or no effect on COX-1 metabolism so that these could be used at sufficiently low doses or at current clinical doses with no adverse side effects.

SUMMARY OF THE INVENTION

The present invention provides a composition comprising~~,~~ as a first ~~component a stilbene~~ genus, and ~~a second component,~~ a compound ~~that specifically and synergistically enhances the~~ anti-inflammatory effect of the first ~~component, a diterpene triepoxide lactone species, a sesquiterpene lactone species, a diterpene lactone species, or a triterpene species. To clarify, there must be~~ a stilbene species as the first component. ~~The second component can be any species selected from the group consisting of a diterpene triepoxide lactone species, a sesquiterpene lactone species, a ditepene lactone species and a triterpene species or derivatives thereof with the proviso that the second component must be different from the first component species~~.

The composition of the present invention must contain, at a minimum~~, two species one each representing the first component and the second component. However, additional species or mixtures of species within the various genera may be present~~ in the composition which is limited in scope only by the ~~combinations of species within the various genera that exhibit the claimed synergistic functionality.~~ The composition functions synergistically to inhibit the activity of inducible COX-2 with little or no effect on COX-1.

The present invention further provides a composition of matter that enhances the function of glucosamine or chondrotin sulfate to normalize joint movement or reduce the symptoms of osteoarthritis.

One specific embodiment of the present invention is a composition comprising an effective amount of ~~resveratrol and at least one compound selected from the group consisting of triptolide, parthenolide, andrographilide, ursolic acid and oleanolic acid.~~

The present invention further provides a method of dietary supplementation and a method of treating inflammation or inflammation-based diseases in an animal which comprises providing to the animal suffering symptoms of inflammation the composition of the present invention containing a ~~second component which specifically and synergistically enhances the anti-inflammatory effect of a stilbene~~ and continuing to administer such a dietary

supplementation of the composition until said symptoms are eliminated or reduced.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

DETAILED DESCRIPTION OF THE INVENTION

Before the present composition and methods of making and using thereof are disclosed and described, it is to be understood that this invention is not limited to the particular configurations, as process steps, and materials may vary somewhat. It is also intended to be understood that the terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

It must be noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

The present invention provides a composition having a specific ~~synergistic~~ inhibitory effect on the activity of COX-2. More particularly, the composition comprises ~~, as a first component, an stilbene, as a second component, at least one member selected from the group consisting of diterpene triepoxide lactones, active sesquiterpene lactones, diterpene lactones, and triterpenes or derivatives thereof as more specifically described above. Preferably, the molar ratio of the active first component to a second component, i.e. the member selected from the group consisting of a diterpene triepoxide lactone species, a sesquiterpene lactone species, a diterpene lactone species, and a triterpene species or~~

~~derivatives thereof is~~ within a range ~~of 1:1 to 1:100, and more preferably~~ within a range ~~of 1:2.5 to 1:10.~~ The composition provided by the present invention can be formulated as a dietary supplement or therapeutic composition. The composition functions ~~synergistically~~ to inhibit the inducibility and/or activity of COX-2 with little or no effect on COX-1.

As used herein, the term "dietary supplement" refers to compositions consumed to affect structural or functional changes in physiology. The term "therapeutic composition" refers to any compounds administered to treat or prevent a disease.

As used herein, the term "CO2 extract" refers to a composition of natural compounds that is capable of inhibiting the activity of COX-2 enzymes or is capable of inhibiting or reducing the severity of a severe inflammatory response.

Therefore, one preferred embodiment of the present invention is a composition comprising ~~-a combination of an effective amount of resveratrol, as a first component, and a second compound selected from the group consisting of triptolide, parthenolide, andrographolide, ursolic acid and oleanolic acid with the proviso that there must be a combination and the first and second component cannot be the same compound, e.g. cannot be the same species within the same genus.~~ The resulting formulation of these combinations functions to synergistically inhibit the inducibility and/or activity of COX-2 while showing little or no effect on COX-1. Therefore, the composition of the present invention essentially eliminates the inflammatory response rapidly without introducing any harmful side effects.

The pharmaceutical grade extract must pass extensive safety and efficacy procedures. Pharmaceutical grade CO2 hops extract refers to a preparation wherein the concentration of As employed in the practice of the invention, the extract has an andrographolide, ursolic acid or oleanolic acid content of about 10 to 95 percent by weight. Preferably, the minimum andrographolide, ursolic acid or oleanolic acid content is greater than 50 percent by weight. The pharmaceutical grade extracts are particularly preferred. A daily dose (mg/kg-

day) of the present dietary supplement would be formulated to deliver, per kg body weight of the animal, about 0.001 to 30 mg CO2 extract of hops.

The composition of the present invention for topical application would contain the following: about 0.001 to 1 wt%, preferably 0.01 to 1 wt% of hops extract.

The preferred composition of the present invention would produce serum concentrations in the following range: ~~0.01 to 10 nM diterpene triepoxid lactones, and 0.001 to 10 µM sequiterpene lacetone, diterpene lactones or triterpenes~~.

In addition to the combination of the active ingredients selected from the group consisting of 30 to 60 percent alpha acids, 10 to 30 percent beta acids, 0 to 6 percent essential oils, 0 to 3 percent water, and 2 to 8 percent fats and waxes the present composition for dietary application may include various additives such as other natural components of intermediary metabolism, vitamins and minerals, as well as inert ingredients such as talc and magnesium stearate that are standard excipients in the manufacture of tablets and capsules.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, isotonic and absorption delaying agents, sweeteners and the like. These pharmaceutically acceptable carriers may be prepared from a wide range of materials including, but not limited to, diluents, binders and adhesives, lubricants, disintegrants, coloring agents, bulking agents, flavoring agents, sweetening agents and miscellaneous materials such as buffers and absorbents that may be needed in order to prepare a particular therapeutic composition. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients, its use in the present composition is contemplated. In one embodiment, talc and magnesium stearate are included in the present formulation. Other ingredients known to affect the manufacture of this composition as a dietary bar or functional food can include flavorings, sugars, amino-sugars, proteins and/or modified starches, as well as fats and oils.

The dietary supplements, lotions or therapeutic compositions of the present invention can be formulated in any manner known by one of skill in the art. In one embodiment, the composition is formulated into a capsule or tablet using techniques available to one of skill in the art. In capsule or tablet form, the recommended daily dose for an adult human or animal would preferably be contained in one to six capsules or tablets. However, the present compositions may also be formulated in other convenient forms, such as an injectable solution or suspension, a spray solution or suspension, a lotion, gum, lozenge, food or snack item. Food, snack, gum or lozenge items can include any ingestable ingredient, including sweeteners, flavorings, oils, starches, proteins, fruits or fruit extracts, vegetables or vegetable extracts, grains, animal fats or proteins. Thus, the present compositions can be formulated into cereals, snack items such as chips, bars, chewable candies or slowly dissolving lozenges.

The present invention contemplates treatment of all types of inflammation-based diseases, both acute and chronic. The present formulation reduces the inflammatory response and thereby promotes healing of, or prevents further damage to, the affected tissue. A pharmaceutically acceptable carrier may also be used in the present compositions and formulations.

According to the present invention, the animal may be a member selected from the group consisting of humans, non-human primates, such as dogs, cats, birds, horses, ruminants or other animals. The invention is directed primarily to the treatment of human beings. Administration can be by any method available to the skilled artisan, for example, by oral, topical, transdermal, transmucosal, or parenteral routes.

The following examples are intended to illustrate but not in any way limit the invention:

EXAMPLE 1

Specific Inhibition of Cyclooxygenase-2 Mediated Prostaglandin E2 by a CO2 Extract of Hops

CLAIMS

We claim:

1. A composition for inhibition of inducible COX-2 activity and having minimal effect on COX-1 activity, said composition comprising an effective amount of alpha acids, beta acids, essential oils, percent water, and fats and waxes.

2. The composition of Claim 1 wherein the hops extract is prepared by CO2 extraction.

3.The composition of Claim 3 wherein the CO2 extract of hops contains 30 to 60 percent alpha acids.

4. The composition of Claim 3 wherein the CO2 extract of hops contains 15 to 45 percent beta acids.

5. The composition of Claim 3 wherein the CO2 extract of hops contains 3 to 6 percent essential oil.

6. The composition of Claim 3 wherein the CO2 extract of hops contains 0 to 3 percent water.

7. The composition of Claim 3 wherein the CO2 extract of hops contains 2 to 8 percent fats and waxes.

8. The composition of Claim ~~8 wherein the sesquiterpene lactone species is parthenolide.~~

10. The composition of Claim 1 wherein the alpha acids are selected from the group consisting of ~~andrographolide, dehydroandrographolide, deoxyandrographolide, neoandrographolide, selenoandrographolide,~~

homoandrographolide, andrographan, amdrographon, andrographosterin, 14-deoxy-11-oxoandrographolide, 14-deoxy-11, 12-didehydroandrographolide, andrographiside, and edelin lactone.

11. The composition of Claim 10 wherein the beta acids are diterpene lactone species is a member selected from the group consisting of andrographolide, dehydroandrographolide, deoxyandrographolide, neoandrographolide, selenoandrographolide, homoandrographolide, andrographan, amdrographon, andrographosterin, 14-deoxy-11-oxoandrographolide, 14-deoxy-11, 12-didehydroandrographolide, and andrographiside.

12. The composition of Claim 11 wherein the diterpene lactone species is a member selected from the group consisting of andrographolide, dehydroandrographolide, deoxyandrographolide, and neoandrographolide.

13. The composition of Claim 12 wherein the diterpene lactone species is andrographolide.

14. The composition of Claim 13 wherein the andrographolide is of pharmaceutical grade.

15. The composition of Claim 1 wherein the triterpene species is a member selected from the group consisting of ursolic acid, oleanolic acid, betulin, betullnic acid, glycyrrhetinic acid, glycyrrhizic acid, triperin, 2 a 3 a dihydroxyurs-12-3n-28-oic acid, 2 a hydroxyursolic acid, 3-oxo-ursolic acid, celastrol, friedelin, tritophenolide, uvaol, eburicoic acid, glycyrrhizin, gypsogenin, oleanolic acid-3-acetate, pachymic acid, pinicolic acid, sopheradiol, soyasapogenol A, soyasapogenol B, tumulosic acid, ursolic acid 3 acetate and sitosterol.

~~16. The composition of Claim 15 wherein the triterpene species is a member selected from the group consisting of ursolic acid, oleanolic acid, betulin, betulinic acid, glycyrrhetinic acid, glycyrrhizic acid, triperin, 2-a-3-a-dihydroxyurs-12-3n-28-oic acid, 2-a-hydroxyursolic acid, 3 oxo ursolic acid, celastrol, friedelin, tritophenolide, and uvaol.~~

~~17. The composition of Claim 16 wherein the triterpene species is a member selected from the group consisting of ursolic acid, oleanolic acid, betulin, betulinic acid, glycyrrhetinic acid, glycyrrhizic acid, and triperin.~~

~~18. The composition of Claim 17 wherein the triterpene species is a member selected from the group consisting of ursolic acid and oleanolic acid.~~

~~19. The composition of Claim 18 wherein the triterpene species is of pharmaceutical grade.~~

~~20. The composition of Claim 1 wherein first and second components are derived from plants or plant extracts.~~

~~21. The composition of Claim 1 wherein at least one of said first or second components is conjugated with a compound selected from the group consisting of mono- or di- saccharides, amino acids, sulfates, succinate, acetate and glutathione.~~

~~22. The composition of Claim 21 wherein said first or second component is conjugated with a compound selected from the group consisting of mono or di- saccharides and amino acids.~~

~~23. The composition of Claim 22 wherein said compound is a mono or di-saccharide and is a member selected from the group consisting of glucose, mannose, ribose, galactose, rhamnose, arabinose, maltose, and fructose.~~

24. The composition of Claim 1, formulated in a pharmaceutically acceptable carrier.

25. The composition of Claim 1, additionally containing one or more members selected from the group consisting of antioxidants, vitamins and minerals.

26. The composition of Claim 1 additionally containing one or more members selected from the group consisting of proteins, fats, carbohydrates, glucosamine, chondrotin sulfate and aminosugars.

27. A method of dietary supplementation in animals comprising administering to an animal suffering symptoms of inflammation a composition comprising effective amount of a first component comprising a member selected from the group consisting of a diterpene triepoxide lactone species and a sesquiterpene lactone species and an effective amount of a second component selected from the group consisting of a diterpene triepoxide lactone species, a sesquiterpene lactone species, a diterpene lactone species, and a triterpene species or derivatives thereof with the proviso that the same diterpene triepoxide lactone species or sesquiterpene lactone species cannot concurrently serve as both said first and second component; and continuing said administration until said symptoms are reduced.

28. The method of Claim 27 wherein the composition is formulated in a dosage form such that said administration provides from 0.001 to 3.0 mg body weight per day of each diterpene triepoxide lactone species, from 0.05 to 5.0 mg body weight per day of each sequesterpene lactone species and from 0.5 to 20.0 mg/kg body weight per day of each diterpene lactone or triterpene species.

29. The method of Claim 27, wherein the composition is administered in an amount sufficient to maintain a serum concentration of 0.1 to 10 nM of each

diterpene triepoxide lactone species; from 0.001 to 10 μM of each sequiterpene lactone species, and from 0.001 to 10 μM of each diterpene lactone or triterpene species..

30. The method of Claim 27 wherein said animal is selected from the group consisting of humans, non human primates, dogs, cats, birds, horses and ruminants.

31. The method of Claim 27 wherein administration is by a means selected from the group consisting of oral, parenteral, topical, transdermal and transmucosal delivery.

32. A method of therapeutic treatment in animals comprising administering to an animal suffering symptoms of arthritis a composition comprising effective amount of a first component comprising a member selected from the group consisting of a diterpene triepoxide lactone species and a sesquiterpene lactone species and an effective amount of a second component selected from the group consisting of a diterpene triepoxide lactone species, a sesquiterpene lactone species, a diterpene lactone species, and a triterpene speciess or derivatives thereof with the provise that the same diterpene triepoxide lactone species or sesquiterpene lactone species cannot concurrently serve as both said first and second component and continuing said administration until said symptoms are reduced.

33. A method of therapeutic treatment comprising applying to the skin of a human suffering symptoms of acne rosacea a lotion comprising a composition comprising effective amount of a first component comprising a member selected from the group consisting of a diterpene triepoxide lactone species and a sesquiterpene lactone species and an effective amount of a second component selected from the group consisting of a diterpene triepoxide lactone species, a sesquiterpene lactone species, a diterpene lactone species, and a triterpene

species or derivatives thereof with the proviso that the same diterpene triepoxide lactone species or sesquiterpene lactone species cannot concurrently serve as both said first and second component and continuing said administration until said symptoms are reduced.

34. A method of therapeutic treatment comprising applying to the skin of a human suffering symptoms of psoriasis a lotion comprising a composition comprising effective amount of a first component comprising a member selected from the group consisting of a diterpene triepoxide lactone species and a sesquiterpene lactone species and an effective amount of a second component selected from the group consisting of a diterpene triepoxide lactone species, a sesquiterpene lactone species, a diterpene lactone species, and a triterpene species or derivatives thereof with the proviso that the same diterpene triepoxide lactone species or sesquiterpene lactone species cannot concurrently serve as both said first and second component; and continuing said administration until said symptoms are reduced.

ABSTRACT

A novel formulation is provided that serves to inhibit the inflammatory response in animals. The formulation comprises 30 to 60 percent alpha acids, 10 to 30 percent beta acids, 0 to 6 percent essential oils, 0 to 3 percent water, and 2 to 8 percent fats and waxes and provides specific inhibition of cyclooxygenase-2 with little or no effect on cyclooxygenase-1.

PROJECT PGE-2 Assay

Notebook No. 2001-07

Continued From Page ____

Experiment 2001-07-04

Purpose: to test activity of PGE2 production in RAW cells with LPS in the presence of the following compounds or combinations.

First plate Week of 4/9/01 - to be read on Thursday 4/12/01

Compound	Function	d1 [ug/mL]	d2 [ug/mL]	d3 [ug/mL]	d4 [ug/mL]	No. Wells
1. Alpha-acids - 166-6-1 Post-run blowdown	Alpha-acids 166-6-1 =	0.125	0.250	0.500	1.000	8
2. Alpha-acids - Original paste	Alpha- acids =	0.063	0.125	0.250	0.500	8
3. Alpha-acids - 166-6-2 Post-run blowdown	Alpha-acids 166-6-2 =	0.125	0.250	0.500	1.000	8
2. Alpha-acids - 166-6-3 Post-run blowdown	Alpha-acids 166-6-3 =	0.125	0.250	0.500	1.000	8
5. Curcuminoids	Curcuminoids =	1.88	3.75	7.50	15.0	8
6. Curcumin	Curcumin =	1.88	3.75	7.50	15.0	8
7. Evodia	Evodia =	3.1	6.3	12.5	25.0	8
					total =	68

Second plate Week of 4/9/01 - to be read on Thursday 4/12/01

Compound	Function	d1 [ug/mL]	d2 [ug/mL]	d3 [ug/mL]	d4 [ug/mL]	No. Wells
1. Diosgenin	Diosgenin =	1.50	3.00	6.0	12.0	8
2. Fisetin	Fisetin =	1.50	3.00	6.0	12.0	8
3. Formononetin	Formononetin =	1.50	3.00	6.0	12.0	8
4. Ipriflavone	Ipriflavone =	1.50	3.00	6.0	12.0	8
5. Kaempferol	Kaempferol =	1.50	3.00	6.0	12.0	8
6. Luteolin	Luteolin =	1.50	3.00	6.0	12.0	8
7. Morin	Morin =	1.50	3.00	6.0	12.0	8
					total =	56

Third plate Week of 4/9/01 - to be read on Thursday 4/12/01

Compound	Function	d1 [ug/mL]	d2 [ug/mL]	d3 [ug/mL]	d4 [ug/mL]	No. Wells
1. Apigenin	Apigenin =	1.50	3.00	6.0	12.0	8
2. Myricetin	Myricetin =	1.50	3.00	6.0	12.0	8
3. Naringenin	Naringenin =	1.50	3.00	6.0	12.0	8
4. Rutin	Rutin =	1.50	3.00	6.0	12.0	8
5. Catechin	Catechin =	1.50	3.00	6.0	12.0	8
6. Trigonellin	Trigonellin =	1.50	3.00	6.0	12.0	8
7. Genistein	Genistein =	1.50	3.00	6.0	12.0	8
					total =	56

Continued on Page 5

Read and Understood By

Signed — Date 4-09-01

Signed — Date 4-5-01

exhibit B

Experiment 2001-07-04

3-96 well plates were set up with 8x10⁴ cells/well RAW cells in 0.2 mL DMEM + 10% FBS + L-glutamine

176 cells were counted

3.52 x 10⁶ Cells in suspension -

~~5.68 ml cell suspension~~ TH

Plating 3 plates with 8 x 10² cells/plate.

8.5 ml cell suspension

~~4.5 ml~~ TH 66.5 mL new media.

To make a total of 75 mLs @ ~~3 x 10⁴~~ TH cells.

Put 25 mL cell suspension into each well of each plate.

4.10.01

Plates were left over night in 37°C 5% CO_2.

Cells looked normal and were 90-95% confluent when treated.

Continued on Page 6

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Signed: [signature] Date: 4.9.01 / 4.10.01

Signed: [signature] Date: 4-15-01

Experiment 2001-07-04

The compounds were weighed out as follows.
Plate 1

Alpha-acids-166-6-1 Ashni # AV1063

$\frac{76.8\text{ mg}}{1000\text{ uL DMSO}} : \frac{.25}{X} = \frac{3.25\text{ mL } 76.8\text{ mg/mL} + 996.75\text{ uL DMSO}}{1\text{mL } 250\text{ ug/mL}}$

Alpha-acids - Ashni # AV1040

$\frac{6.2\text{mg}}{1000\text{uL}} : \frac{.125}{X}$ $X = 20.16\text{ uL}$

$\text{DMSO} = 979.8\text{ uL DMSO}$

$\frac{}{1\text{mL } 125\text{ug/ml}}$

Alpha-Acids-166-6-2 Ashni # AV1064

$\frac{33.2\text{mg}}{1000\text{uL}} : \frac{.25}{X}$ $X = 7.5\text{ uL } 33.2\text{mg/ml}$

$\frac{979.8\text{ uL DMSO}}{1\text{mL } 250\text{ uL/mL}}$

Apha-Acids-166-6-3 Ashni # AV1065

$\frac{24.4\text{mg}}{1000} : \frac{.25}{X}$ $X = 10.25\text{ uL}$

$\frac{989.75\text{ uL DMSO}}{1\text{mL } 250\text{ uL/ml}}$

Curcominoids Ashni # AV1060

$\frac{3.15}{1000} = \frac{3.5}{X}$ $X = 933.3\text{ uL DMSO} = 3.75\text{ mg/ml}$

Curcumin Sigma C-1386 Lot 69H3457

$\frac{3.75}{1000} = \frac{3}{X}$ $X = 800\text{ uL DMSO} = 3.75\text{ mg/ml}$

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Signed 4.10.01 Date

Signed 4-15-01 Date

Experiment 2001-07-04

Evodia Ashni # AV1062

$\frac{6.25}{1000} = \frac{5.4}{x}$ $x = 864$ ul DMSO = 6.25 mg/ml

Plate 2.

Diosgenin Sigma D-1634 Lot 89H1221

$\frac{3}{1000} = \frac{2.6}{x}$ $x = 533.3$ ul DMSO = 3 mg/ml

Fisetin Sigma F4043 Lot 60K1869

$\frac{3}{1000} = \frac{2.4}{x}$ $x = 800$ ul DMSO = 3 mg/ml

Formononetin
Aldrich 47752 Lot 37148811 43300

$\frac{3}{1000} = \frac{2.7}{x}$ $x = 900$ ul DMSO = 3 mg/ml

Ipriflavone Fisher 342470010 Lot AD1343501

$\frac{3}{1000} = \frac{2.4}{x}$ $x = 866.66$ ul DMSO = 3 mg/ml

Kaempferol Sigma K-033 Lot 129H1017

$\frac{3}{1000} = \frac{1.5}{x}$ $x = 500$ ul DMSO = 3 mg/ml

Luteolin Sigma L-9283 Lot 11K4085

$\frac{3}{1000} = \frac{2.4}{x}$ $x = 800$ ul DMSO = 3 mg/ml

Continued on Page 8

Read and Understood By

Signed 4-10-01 Date

Signed 4-15-01 Date

Experiment 2001-07-04

Morin Sigma M-4008 Lot 10K2502

$\frac{3}{1000} = \frac{3}{x}$ $x = 1000$ uL DMSO = 3mg/ml

Plate 3

Apigenin Sigma A-3145 Lot 60K0780

$\frac{3}{1000} = \frac{2.1}{x}$ $x = 700$ uL DMSO = 3mg/ml

Myricetin Sigma M-6766 Lot 99H2503

$\frac{3}{1000} = \frac{1.3}{x}$ $x = 433.33$ uL DMSO = 3mg/ml

Naringenin Sigma N-5893 Lot 79H0547

$\frac{3}{1000} = \frac{2.5}{x}$ $x = 833.33$ uL DMSO = 3mg/ml

Rutin Sigma R-5143 Lot 10K0177

$\frac{3}{1000} = \frac{3}{x}$ $x = 1000$ uL DMSO = 3 mg/ml

Catechin Sigma C-1251 Lot 60K1376

$\frac{3}{1000} = \frac{1.7}{x}$ $x = 566.66$ uL DMSO = 3mg/ml

Trigonellin Sigma T-5509 Lot 29H1264

$\frac{3}{1000} = \frac{1.9}{x}$ $x = 566.66$ uL DMSO = 3mg/ml

Genistein Sigma G-6776 Lot 100K4020

$\frac{3}{1000} = \frac{1.2}{x}$ $x = 400$ uL DMSO = 3mg/ml

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Read and Understood By

J. Crawford 4.10.01
Signed Date

Signed 4-15-01
Signed Date

Experiment 2001-07-04

Each treatment was then a 250x DMSO Stock

DMSO is by Labcard-5507

For each treatment 3 serial dilutions were done by taking 20uL stock + 200uL DMSO. The dilutions were done as outlined on p. 4 of this notebook.

For each treatment 1 microfuge tube was set with 1 mL SerumFree DMEM + P/S
DMEM - Cellgro #10-013-CV Lot 10013280
PenStrep - Cellgro #30-001-CI Lot 30001069
To each tube 4uL of the DMSO stock was added. Each treatment was then at the concentration stated on p. 4.
- 200uL in duplicate was added to a 96 well plate
- Each plate was then equilibrated in the incubator at 37°C 5% CO_2 for 10 min.

	Dilution 1*	Dilution 2	Dilution 3	Dilution 4
Columns	1 and 2	3 and 4	5 and 6	7 and 8	9 and 10	11 and 12

Compound/Combo #		1	2	3	4	5	6
1	A	Positive Control	Negative Control	Dilution 1*	Dilution 2	Dilution 3	Dilution 4
2	B	"	"	Dilution 1*	Dilution 2	Dilution 3	Dilution 4
3	C	"	"	Dilution 1*	Dilution 2	Dilution 3	Dilution 4
4	D	"	"	Dilution 1*	Dilution 2	Dilution 3	Dilution 4
5	E	"	"	Dilution 1*	Dilution 2	Dilution 3	Dilution 4
6	F	"	"	Dilution 1*	Dilution 2	Dilution 3	Dilution 4
7	G	"	"	Dilution 1*	Dilution 2	Dilution 3	Dilution 4
Untreated Cells	H						

Continued on Page 10

Read and Understood By

Signed — Date: 4.10.01

Signed — Date: 4-15-07

Experiment 2001-07-04

- 100ul media was removed from All the Cellwells.
- 100ul of the treatments was the added back to these wells.
- These final plates were then incubated for 90minutes at 37°C 5% CO2
- Prepair LPS- 44ul of 1mg/ml Stock into 10mL DMEM (Serumfree) Add 20ul to each well except neg- control.

4.11.01
Cell observation:
Alpha-acids 166-6-3 Post-mix bloudown was not as confluent as the other cells.
But overall for plate 1 notoxicity noted.
No visual toxicity noted for plate 2.
No visual toxicity noted for plet 3.

- Supernates were collected for each treatment and the negative and positive controls
- Perform Cell viability Assay
  - Using Calcein dye by Molecular Probes a 1mg/ml solution C-3099 Lot 34951-24
- Wash each plate 2X w/ warm PBS, 400ul/well/wash
- Prepare Calcein working solution
  Add 40ul Calcein to 20mL warm PBS to make 2mM working solution.

Continued on Page 11

Read and Understood By

Signed 4.10.01 4.11.01 Date
Signed 4-15-01 Date

Experiment 2001-07-04

- Add 100 ul working solution/well
- incubate 35 minutes @ RT.
- Read Plate in florometer.
- In row H1 - H6 all media was removed and treated w/ 70% MeOH for 20minutes then washed and treated w/ working solution.

Raw Data for cell viability assay

Wed Apr 11 16:36:52 2001 PlateReader 3.0 for Windows Page 1
Instrument: FluoroCount with Filter Wheels Serial #: 424341 Institution: Ariad

Plate File: c:\reader\detailive.pf Created: Wednesday, January 10, 2001 2:52:33 PM
Plate File Description: Plate file description

Data Set Information

Plate ID: 49, 1/1
Comment:

Read Settings

Read at: Wednesday, April 11, 2001 4:08:10 PM PMT: 1091 volts Max RFU Well: B12
Excitation Filter: 485nM Gain Level: 1.0, auto
Emission Filter: 530nM Read Length (sec): 0.5
Plate Type: Generic 96 well

Raw Data

Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	52318	47231	37794	37844	47471	45584	46456	45710	45343	43967	45164	52292
B	44688	41501	32364	31250	41277	39715	40909	40720	40813	41027	42267	54600
C	45410	40758	30858	28773	39922	36406	37811	37602	38979	39704	40138	50784
D	41208	41473	30158	28734	37442	34280	37411	38494	38668	38503	39725	52862
E	46297	41564	28609	27992	38059	35754	36998	35874	35617	37448	40977	52328
F	40742	39246	28209	28000	38559	38067	39463	37101	38731	37686	39528	52410
G	42006	39151	29585	29700	34588	38390	38520	41445	38584	40050	41504	51959
H	1629	3301	3273	3950	3215	3350	35607	37510	39894	39058	41380	46107

Wed Apr 11 16:36:17 2001 PlateReader 3.0 for Windows Page 1
Instrument: FluoroCount with Filter Wheels Serial #: 424341 Institution: Ariad

Plate File: c:\reader\detailive.pf Created: Wednesday, January 10, 2001 2:52:33 PM
Plate File Description: Plate file description

Data Set Information

Plate ID: 50, 1/1
Comment:

Read Settings

Read at: Wednesday, April 11, 2001 4:12:05 PM PMT: 1098 volts Max RFU Well: C12
Excitation Filter: 485nM Gain Level: 1.0, auto
Emission Filter: 530nM Read Length (sec): 0.5
Plate Type: Generic 96 well

Raw Data

Plate 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	56718	53788	43075	43648	52589	49058	48362	42109	48044	47390	45918	55534
B	51135	44709	34204	34771	45757	41959	45068	45024	41556	41973	32342	44758
C	40070	42211	35306	32031	43760	40107	38573	41076	39424	40902	37570	55492
D	47829	41538	31286	31593	39568	36726	38172	38342	39855	39716	42778	49769
E	46760	45061	31656	31225	39539	37242	36456	36131	33896	36431	29872	38591
F	44651	40725	30265	29243	36902	36315	35827	35978	31895	34218	34560	30210
G	38459	36782	27698	28971	38107	36314	37957	28067	39145	40512	40018	40359
H	3441	3203	3983	3503	3292	3304	32213	29964	33640	35052	30495	33330

Wed Apr 11 16:36:01 2001 PlateReader 3.0 for Windows Page 1
Instrument: FluoroCount with Filter Wheels Serial #: 424341 Institution: Ariad

Plate File: c:\reader\detailive.pf Created: Wednesday, January 10, 2001 2:52:33 PM
Plate File Description: Plate file description

Data Set Information

Plate ID: 51, 1/1
Comment:

Read Settings

Read at: Wednesday, April 11, 2001 4:22:50 PM PMT: 1046 volts Max RFU Well: A02
Excitation Filter: 485nM Gain Level: 1.0, auto
Emission Filter: 530nM Read Length (sec): 0.5
Plate Type: Generic 96 well

Raw Data

Plate 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	37702	36395	48656	49237	50836	50590	43511	41919	40192	40127	31722	27712
B	51972	60042	41299	40762	40456	44481	45319	44292	42359	41933	41049	47225
C	50460	45385	41461	40509	45100	45165	42935	42941	40715	40655	38962	48105
D	50775	46151	41992	39722	44721	42054	43244	42927	40718	40244	41160	51501
E	47358	48243	39922	37752	43348	41977	41844	42794	42045	44511	43159	55309
F	47764	43675	40559	39315	40522	41662	41555	39170	40505	40434	41802	47914
G	45064	46509	39007	40327	42136	40578	40925	40008	37084	37649	33057	52053
H	2081	2519	2500	2513	2473	2351	41017	45063	42049	42811	40695	23674

Continued on Page 12

Signed [signature] Date 4-11-01

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Signed [signature] Date 4-15-01

Experiment 2001-07-04

- Take the collected supernates and use a 96 well plate to make 1:10 dilutions of each compound @ each dose.

20ul supernate + 180ul Fresh DMEM
This is for each dilution and the negative control.

For the positive control use 3 dilutions
1:10 - 20ul Pos + 180ul Fresh DMEM
1:50 - 20ul Pos + 980ul Fresh DMEM
1:100 - 10ul Pos + 990ul Fresh DMEM
Use these supernate dilutions and the SOP on Page 1+2 of this notebook to set up the following plates.

Plate 1, 4.12.01

	1	2	3	4	5	6	7	8	9	10	11	12
A	S	TA	51 (1000)	33	LPS+ 1:10							
B	S	TA	51	35	LPS+ 1:50							
C	S	950	32	37	LPS+ 1:100							
D	S	950	32	36	LPS+ 1:10							
E	S	Bo	33	32	LPS+ 1:50							
F	S	Bo	33	34	LPS+ 1:100							
G	S	Bo	34	35	LPS -							
H	S	Bo	34	33 (7.8)	LPS -							

Plate 2, 4.12.01

	1	2	3	4	5	6	7	8	9	10	11	12
A	S	TA	51 (1000)	35	LPS+ 1:10							
B	S	TA	51	35	LPS+ 1:10							
C	S	950	32	37	LPS+ 1:50							
D	S	950	32	36	LPS+ 1:50							
E	S	Bo	33	37	LPS+ 1:100							
F	S	Bo	33	37	LPS+ 1:100							
G	S	Bo	34	36	LPS -							
H	S	Bo	34	36 (7.8)	LPS -							

Plate 3, 4.12.01

	1	2	3	4	5	6	7	8	9	10	11	12
A	S	TA	51 (1000)	35	LPS+ 1:10							
B	S	TA	51	35	LPS+ 1:10							
C	S	950	32	37	LPS+ 1:50							
D	S	950	32	36	LPS+ 1:50							
E	S	Bo	33	37	LPS+ 1:100							
F	S	Bo	33	37	LPS+ 1:100							
G	S	Bo	34	36	LPS -							
H	S	Bo	34	36 (7.8)	LPS -							

Continued on Page 13

Read and Understood By

Signed 4.11.01 Date

Signed 4-15-01 Date

Experiment 2001-07-04

After plates incubated @ 40c for 18 hours they were washed 5X w/ wash buffer. Developer was added. The RAW data is as follows.

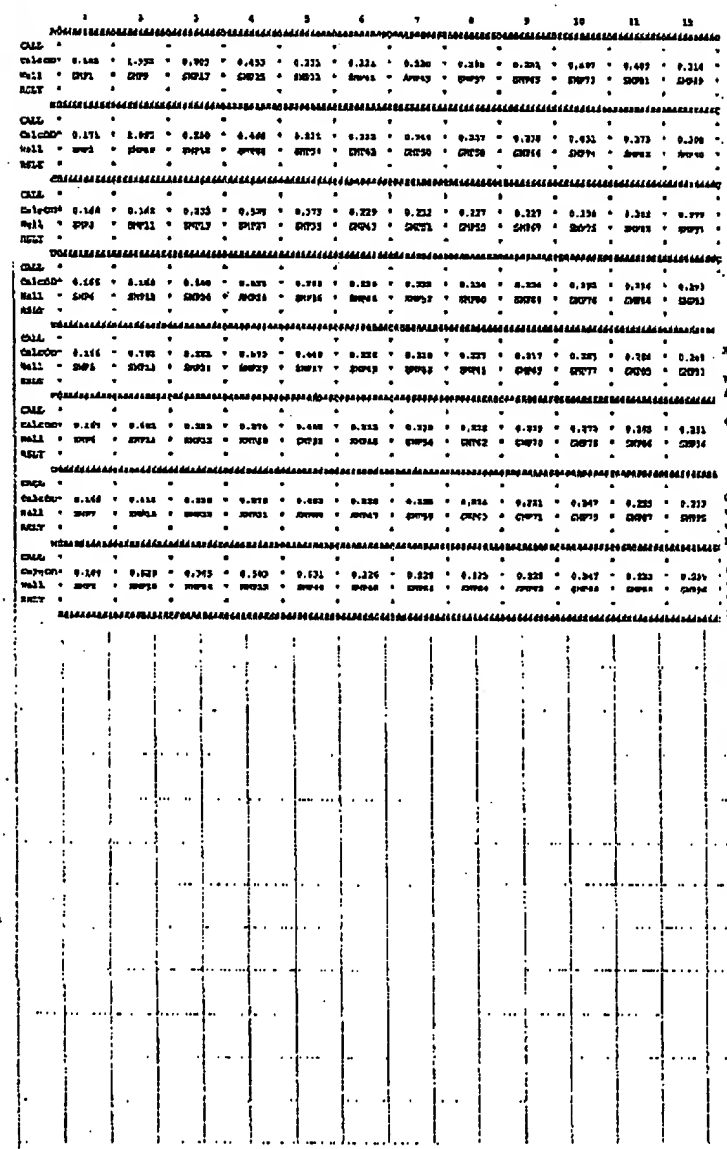
Bio-Tek Instruments

Assay: Quick Read Date: 04/12/01 Lot: plate 1

Wavelength: 405 Time: 01:13:58PM Operator:

Temp: Plate ID:

COMMENTS



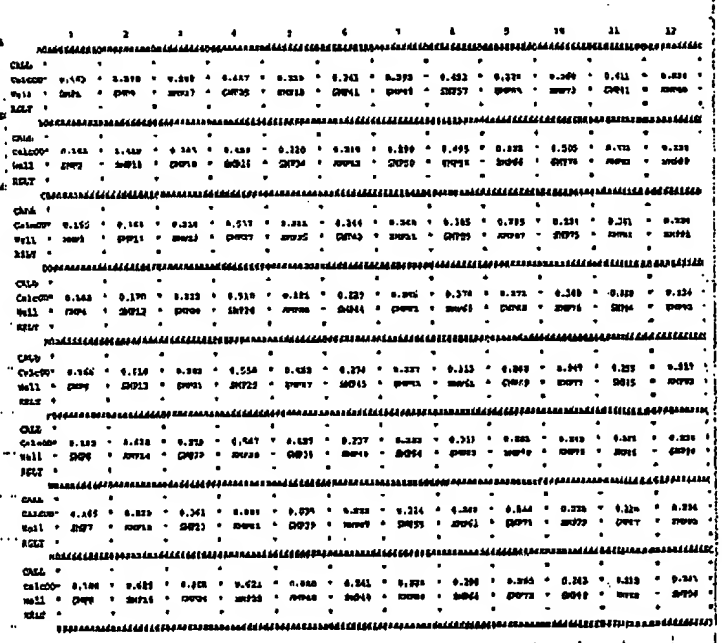
Bio-Tek Instruments

Assay: Quick Read Date: 04/12/01 Lot: plate 2

Wavelength: 405 Time: 01:15:34PM Operator:

Temp: Plate ID:

COMMENTS



Continued on Page 14

Read and Understood By

Signed — 4.12.01 Date

Signed — 4-15-01 Date

PROJECT PGE$_2$ Assay

Notebook No. 2001-07
Continued From Page 13

Experiment 2001-07-04

Bio-Tek Instruments

Assay: QUICK Read | Date: 04/12/01 | Lot: plate 3
Time: 03:17:13PM | Operator:
Wavelength: 405 | Temp: | Plate ID:

COMMENTS

	1	2	3	4	5	6	7	8	9	10	11	12
CalcOD	0.188?	[illegible]	0.153	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.219?	[illegible]
Well	[illegible]	[illegible]	[illegible]	SMP15?	GMP33?	GMP41?	GMP49?	GMP57?	[illegible]	[illegible]	[illegible]	[illegible]
CalcOD	0.199?	1.333	[illegible]	[illegible]	0.323?	0.326?	0.307?	0.230?	0.214?	0.210?	[illegible]	[illegible]
Well	[illegible]	[illegible]	SMP10?	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CalcOD	[illegible]	0.176?	0.241?	0.575?	[illegible]	[illegible]	[illegible]	0.235?	[illegible]	[illegible]	[illegible]	[illegible]
Well	[illegible]	SMP11?	GMP19?	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CalcOD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Well	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CalcOD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.239?	[illegible]
Well	[illegible]	[illegible]	[illegible]	[illegible]	GMP37?	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	SMP15?	[illegible]
CalcOD	[illegible]	[illegible]	0.270?	[illegible]	[illegible]	[illegible]	0.223?	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Well	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CalcOD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Well	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
CalcOD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Well	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Raw data plate 3.

4-15-01

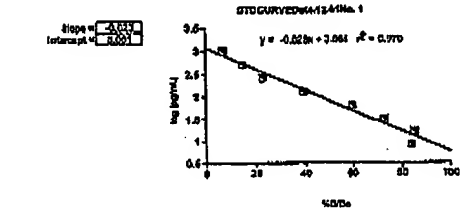
Continued on Page

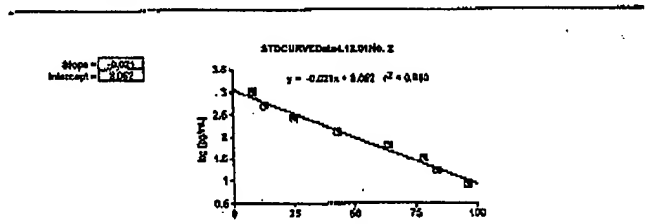
[signature] Signed | 4-12-01 Date

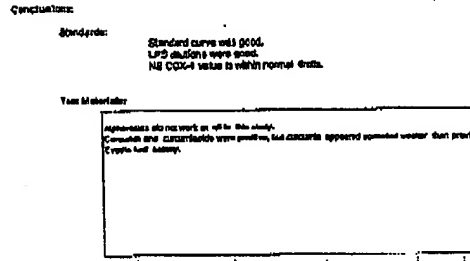
Read and Understood By

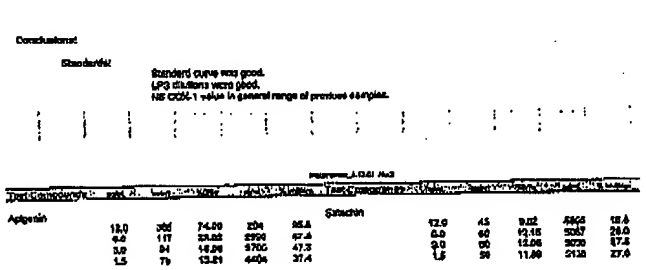
[signature] Signed | 4-15-01 Date

2001-07 + 04









Conclusions:

Standards:

Standard curve was good.
LPS dilutions were good.
NS COX-1 value is within normal limits.

Test Materials:

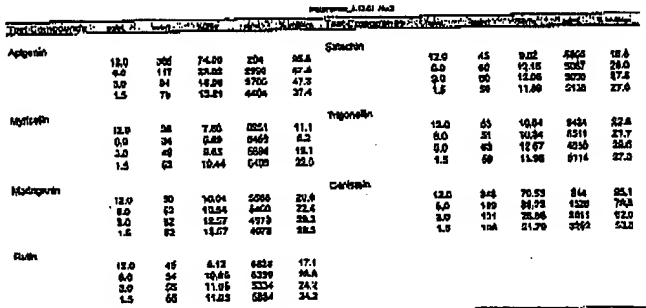
Compounds did not work at all in this study.
Curcumin and curcumicin were positive, but curcumin appeared somewhat weaker than previous results have shown.
Crypto had activity.

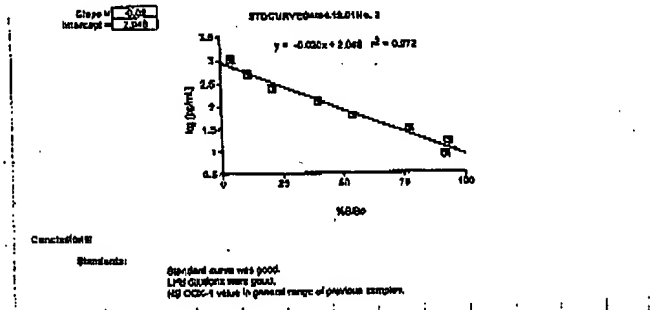
Conclusions:

Standards:

Standard curve was good.
LPS dilutions were good.
NS COX-1 value in general range of previous samples.

+Calculated data





Conclusions:

Standards:

Standard curve was good.
LPS dilutions were good.
NS COX-1 value in general range of previous samples.

Continued on Page 16

Read and Understood By

Signed

4-16-01

Date

Signed

5-5-01

Date

Phone Log 4
January 2001 to August 2001

[REDACTED]

5/2/01

- Message from [POTENTIAL BUSINESS PARTNER]. Said he represents company that can do microencapsulation of raw materials.

5/9/01

- [POTENTIAL BUSINESS PARTNER] wants to work on our hops product. Will fax him a CDA. Called [ASHNI ADMINISTRATIVE ASSISTANT] to fax me a 2-way CDA. She will do this.
- Called [SUPPLIER] to order 1 kg of CO2 extract to be shipped to [POTENTIAL BUSINESS PARTNER].
- Called [POTENTIAL BUSINESS PARTNER] and left message that the fax number he gave me was not working to send CDA.

5/10/01

- Got fax number from [POTENTIAL BUSINESS PARTNER] to send CDA

5/14/01

- Meeting at [POTENTIAL MANUFACTURER] to discuss manufacturing in general. As one topic the manufacturing of hops capsules was discussed.

5/16/01

- [POTENTIAL BUSINESS PARTNER] called to discuss one item on the CDA.

5/21/01

- [POTENTIAL BUSINESS PARTNER] called and was interested in receiving some data and discussed the business model for a joint venture.

5/22/01

- [POTENTIAL BUSINESS PARTNER] called "needs complete package."

5/31/01

- [POTENTIAL BUSINESS PARTNER] called and gave address to send data package.
- [POTENTIAL BUSINESS PARTNER] message said he could not open powerpoint presentation that I emailed him.
- [POTENTIAL BUSINESS PARTNER] call returned; discussed retail cost of hops CO2 extract.

6/5/01

- Left message with [POTENTIAL BUSINESS PARTNER]; [ASHNI ADMINSTRATIVE ASSISTANT] opened powerpoint file easily.

6/6/01

- Called [POTENTIAL BUSINESS PARTNER] and asked about supply of microencapsulated material. He stated that when completed it will contain 80 to 90% of starting material. Very little dilution of starting material.

6/7/01

- [POTENTIAL BUSINESS PARTNER] called to say that he will call tomorrow with discussion points on joint venture.

6/8/01

- Call with [POTENTIAL BUSINESS PARTNER] to discuss joint venture

EXHIBIT C

[REDACTED]

6/14/01

- Working on the hops/COX-2 patent can finish in the next several hours.

6/15/01

- Message from [POTENTIAL BUSINESS PARTNER]

6/15/01

- hops patent application sent to [PATENT COUNSEL]; will complete hops synergy by Wednesday.
- Called [POTENTIAL BUSINESS PARTNER] and discussed results of hops testing so far; told him I needed something from his side on the proposed terms of the joint venture. Mentioned that I spoke with [POTENTIAL SUPPLIER] for source of hops extract. [POTENTIAL BUSINESS PARTNER] mentioned that the glucosamine market was $100 M and we could get a large share of this with a fast acting product containing hops and glucosamine. Patent for glucosamine was granted in 1961, so there is no protection any longer.

6/19/01

- [POTENTIAL BUSINESS PARTNER] called discussed stratification of products e.g. glucosamine+hops, collagen+hops, for different customers. Indicated price of processing would be $12/kg.

6/20/01

- [POTENTIAL BUSINESS PARTNER] called and discussed doses of hops (CO_2 extract) that would be required with glucosamine formulation.
- [PATENT COUNSEL] called to say we are ready to file.

[REDACTED]

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